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Original Article

Variants in C-reactive protein and IL-6 genes and susceptibility to obstructive sleep apnea in children: a candidate-gene association study in European American and Southeast European populations

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ABSTRACT

Background: Preliminary evidence indicates that variants of the C-reactive protein (CRP) and IL-6 genes might be associated with the presence of obstructive sleep apnea (OSA) in childhood. Thus a candidate-gene association study was conducted to investigate the association of four variants of the CRP gene (1444C/T, −717T/C, 1861C/T, and 1919A/T) and two variants of the IL-6 gene (−174G/C and 597G/A) with OSA in a cohort of European American and Greek children.
Methods: The genetic risk effects were estimated based on the odds ratio (OR) of the allele contrast and the generalized odds ratio (OR_G), which is a model-free approach. The mode of inheritance was assessed using the degree of dominance index. The impact of haplotypes was also examined.
Results: In the American population, the allele contrast and the model-free approach produced significant ORs for the CRP 1444C/T variant (OR, 3.82 [95% confidence interval {CI}, 1.91–7.63] and OR_G, 4.37 [95% CI, 1.96–9.76]), respectively, and the mode of inheritance was recessiveness of allele T. Significance was also shown for the CRP 1919A/T variant (OR, 2.45 [95% CI, 1.23–4.85] and OR_G, 2.76 [95% CI, 1.26–6.03]) with the mode of inheritance being nondominance of allele T. For the IL-6-174G/C variant, there was an indication of recessiveness of allele C. Finally, the IL-6-174C/IL-6 597A haplotype was associated with OSA. In the Greek population, no association was detected for any variant or haplotype.
Conclusions: Genetic variation in the IL-6/CRP pathway was associated with increased risk for OSA in European American children and may account for the higher CRP levels in the context of pediatric OSA compared to Greek children.

1. Introduction

Obstructive sleep apnea (OSA) in childhood is a syndrome of functional impairment of the upper airway during sleep [1]. Airway patency is maintained by complex interactions between upper airway resistance, pharyngeal collapsibility, tone of the pharyngeal dilator muscles, and negative intraluminal pressure generated by the muscles of inspiration [2]. Disorders affecting components of the upper airway, such as adenotonsillar hypertrophy, special craniofacial characteristics, or abnormal neuromotor tone, can disrupt this fine balance of mechanical forces and may predispose to apneas and hypopneas in a sleeping individual [3]. Aggregation of OSA cases within families and results of multipoint variance-component linkage analysis indicate that genetic variants in addition to environmental influences are associated with an increased risk for upper airway dysfunction during sleep [4,5].

Previous research efforts were focused on the identification of gene polymorphisms potentially involved in pathways

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intermediate to OSA, such as upper airway neuromotor tone, obesity, and inflammation, and the results were inconclusive [6]. In a recent candidate-gene study by Larkin et al. [7] of 1423 European and African American children and adults from families with or without OSA index cases, 53 genes potentially involved in the pathogenesis of upper airway dysfunction were assessed. The authors suggested that upper airway obstructive events may trigger an inflammatory cascade, but inflammatory processes could also predispose to OSA [7]. Significant associations were identified between single nucleotide polymorphisms (SNPs) of C-reactive protein (CRP) (5237A/G-rs2808630) or glial cell–derived growth factor genes and OSA in European Americans [7]. CRP is a marker of systemic inflammation and glial cell–derived growth factor is implicated in ventilatory control pathways. Regulation of CRP expression mostly occurs at the transcriptional level, with IL-6 being the major inducer [8]. Episodic hypoxemia in participants with OSA increases oxidative stress and activates redox-sensitive transcription factors (e.g., nuclear factor κB and hypoxia inducible factor 1), which enhance the release of proinflammatory mediators such as IL-6 [9]. An association between severity of OSA with plasma CRP levels has been demonstrated in American children, and it has not been replicated in a cohort of Greek children implying different genetic or environmental influences in these two populations [10,11].

In our present candidate-gene study, we evaluated the potential association of four CRP gene variants (1444C/T, −717T/C, 1861C/T, and 1919A/T) and two IL-6 variants (−174G/C and 597G/A) with the presence of OSA in two different populations: a European American cohort and a Southeast European (Greek) cohort. Analysis of gene haplotypes and estimation of the mode of inheritance were performed [12,13]. In addition, a genetic model-free approach was adopted [13,14].

2. Methods

2.1. Study population

2.1.1. European American cohort

Consecutive children who were referred to the University of Louisville Pediatric Sleep Medicine Center for evaluation of habitual snoring and suspected OSA were recruited after obtaining informed consent from their legal caretaker. In addition, community-based data were collected for our study, which was approved by the University of Louisville Human Research Committee and the Boards of the participating schools (Jefferson County Public Schools and Archdiocese of Louisville Catholic Schools) from which the community sample was recruited.

To screen for eligibility of the participants, parents filled out a validated sleep questionnaire [15]. On the basis of the completed questionnaire, both nonsnoring and snoring children were randomly selected and invited to participate in the study. Exclusion criteria for participation in the study were: (i) chronic medical conditions, (ii) genetic or craniofacial syndromes, (iii) developmental delays, and (iv) presence of acute or chronic infections. Children who did not meet exclusion criteria were invited to the Sleep Disorders Laboratory for overnight polysomnography (PSG). For our study, only children who answered Caucasian (white) to the ethnicity question were retained.

2.1.2. Greek cohort

Children with or without habitual snoring (present >3 nights/week) were recruited and underwent PSG at the Larissa University Hospital Sleep Disorders Laboratory. Exclusion criteria for participation in the study were identical to those applied for the American cohort. The study protocol was approved by the Larissa University Hospital Ethics Committee. Parental informed consent and child assent were obtained.

The study design was equivalent in both centers and recruitment of participants was initiated in January 2007; it progressed prospectively and was completed 18 months later. In all participants of both cohorts, weight and standing height were measured and body mass index (BMI) z scores were calculated. Participants with BMI z scores of $\geqslant 1.645$ (95th percentile) were defined as obese. Calculation of BMI z score was based on the Centers for Disease Control growth charts [16,17].

2.2. PSG

All children underwent overnight PSG in the Sleep Disorders Laboratory of each center, as previously described [18,19]. Identical definitions of sleep events and PSG scoring methods were used in both centers [19,20]. Obstructive apnea was defined as the absence of airflow for at least two breaths in duration in the presence of chest/abdominal wall motion. Hypopnea was defined as a reduction in the airflow signal amplitude of at least 50% compared to baseline, in the presence of chest/abdominal wall motion and in association with oxygen desaturation of hemoglobin equal to or greater than 4% or with an electroencephalographic arousal. The obstructive apnea–hypopnea index (OAHI) was calculated as the sum of obstructive and mixed apneas (apneas with both central and obstructive component) and hypopneas per hour of total sleep time. OSA was defined as an OAHI >1 episode per hour [1]. PSG scoring was identical in both centers and has been validated in a previous study performed by both research groups [20].

2.3. Laboratory assays

Fasting peripheral blood samples were collected in vacutainer tubes containing ethylenediaminetetraacetic acid (Becton Dickinson, Franklin Lakes, NJ). All DNA samples were extracted using QIAmp DNA blood kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Concentration and quality of the DNA were determined by a ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE). The purity of the DNA was assessed by calculating the ratio of absorbance at 260/280 nm, and all DNA samples were found to have a ratio of 1.8–1.9. The precise length of genomic DNA was determined by gel electrophoresis with 1% agarose gel. All of the purified samples were stored at −80 °C until further analyses.

Genotyping was performed using the ABI PRISM 7500 Sequence Detection System for allelic discrimination following the manufacturer's instructions (Applied Biosystems; Foster City, CA). Four SNPs in the human CRP gene (1444C/T [rs1130864]; −717T/C [rs2794521]; 1861C/T [rs1205]; 1919A/T [rs1417938]) and two SNPs in the IL-6 gene (−174G/C [rs1800795]; and 597G/A [rs1800797]) were selected for our genetic association study. All six SNPs were established in previous studies and their associations with other outcomes have been previously explored [21–26] or had a minor allele frequency greater than 18% based on information in the National Center for Biotechnology Information SNP Database and the Applied Biosystems-Celera Discovery System Database (http://www.appliedbiosystems.com).

Detection of the four SNPs of the CRP gene and two SNPs of IL-6 gene were completed by TaqMan technology (Applied Biosystems). Two fluorogenic minor groove binder probes were used for each locus with the dyes 6-carboxyfluorescein (FAM; excitation, 494 nm) and VIC (excitation, 538 nm), which are easily differentiated in real-time polymerase chain reaction systems.

Realtime polymerase chain reaction was performed using 12.5 μL of TaqMan 2× universal master mix (Applied Biosystems), 1.25 μL of each primer, 10.25 μL of RNase and DNase-free water (Ambion; Austin, TX), and 1 μL of sample DNA in a total volume

of 25 μL per single-well reaction. Two wells of a 96-well plate (Applied Biosystems) were used for each sample and for each of the six SNPs. DNase-free water was included in each assay, run as non-template control. Assay conditions were 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Initially, the SNP assay was set up using SDS software-version 2.1 (Applied Biosystems) as an absolute quantification method, but the plate was read using the allelic discrimination settings after assay completion. Genotyping was performed by laboratory personnel blinded to clinical status.

2.4. Data analysis

The European American and Greek case group and control participants were compared regarding age, BMI *z* score, and frequency of obesity or male gender using unpaired *t* tests for continuous variables and χ^2 tests for categorical characteristics. The association between genotype distribution and clinical status was tested using the χ^2 test. The codominant and additive models of the cases were compared to the control group using a logistic model. The significance of the genetic models was also tested using the Fisher's exact test. The comparison associations were expressed in odds ratios (ORs) unadjusted and adjusted for age, gender, and obesity with the corresponding 95% confidence interval (CI).

The associations were also examined using the generalized odds ratio (OR_G) [14]. The OR_G is a genetic model-free approach and provides an estimate of the overall risk effect by utilizing the complete genotype distribution. The OR_G expresses the probability of a participant being diseased relative to the probability of being nondiseased, given that the diseased participant has a higher mutational load than the nondiseased. The testing of a genetic association using the OR_G is powerful even in the absence of Hardy–Weinberg equilibrium (HWE) [27].

In control participants, deviation of the genotype distribution from HWE and existence of linkage disequilibrium (LD) between polymorphisms were evaluated using exact tests according to Weir [28]. The ORs were recalculated after correction for deviation from HWE for the gene variants within control participants who deviated from it [29]. The mode of inheritance was estimated using the degree of dominance index (*h*-index) [12,13]. LD and haplotypes were tested only for variants with the control participants in HWE. A result was considered statistically significant when $P < 0.05$.

The unadjusted and adjusted ORs were calculated using SPSS version 11.5 (SPSS Inc; Chicago, IL, USA). HWE and LD were tested using Genetic Data Analysis [30]. The haplotype frequencies were estimated and compared by SHEsis [31]. OR_G was calculated using ORGGASMA software [14].

3. Results

3.1. Characteristics of the participants and distribution of gene variants

Characteristics of the cases and the control groups for the two populations are summarized in Table 1. In European American children, participants with OSA had a significantly higher frequency of obesity compared to participants without OSA ($P = .03$). In addition, controls in the Greek population were significantly older relative to cases ($P = .03$). European American and Greek controls did not differ in age, BMI *z* score, and frequency of obesity or male gender ($P > .05$). European American and Greek cases differed significantly only in age ($P = .03$).

Distribution of the CRP 1444C/T, CRP −717T/C, CRP 1861C/T, CRP 1919A/T, IL-6-174G/C, and IL-6 597G/A variants among cases and control subjects for the European American and Greek children are shown in Table 2. In the European American population, control participants did not conform to HWE for the variants CRP 1444C/T and CRP 1919A/T ($P < .01$ and $P = .01$, respectively). Similarly, control participants deviated from HWE for the CRP −717T/C variant in the Greek population ($P = .03$).

3.1.1. European American children

Significant association between clinical status (OSA/no OSA) and genotype distribution was shown for the SNPs CRP 1444C/T, CRP 1919A/T, and IL-6-174G/C (Table 2). For the CRP 1444C/T variant, the allele contrast and the model-free approach produced significant ORs (OR, 3.82 [95% CI, 1.91–7.63]) and OR_G, 4.37 [95% CI, 1.96–9.76], respectively [Table 3]). The model free approach indicated that for any two subjects, one with OSA and one without OSA, the odds of being with OSA was 4-fold higher than the odds of being without OSA, given that the subject with OSA has higher mutational load than the one without OSA. Alternatively, there were four times as many diseased–healthy (with OSA–without OSA) pairs in the study for which the diseased had the largest mutational load as there were pairs for which the healthy had the largest mutational load. Subjects who were homozygous for T allele were considered to have the highest mutational load, those homozygous for C allele to have the lowest, and those who were heterozygous to have an intermediate load. The additive and codominant models also produced significant ORs (unadjusted or adjusted for age, gender, and obesity, and corrected for deviation from HWE [OR, 15.8 {95% CI, 3.94–63.43} and OR, 2.47 {95% CI, 1.17–5.21}, respectively]; Table 3). The mode of inheritance, after correcting for deviation from HWE, was recessiveness of allele T ($h = 1.15$) (Table 4).

Table 1
Characteristics of participants for the case group and control group in European American and Greek children.

Population	Parameters	Case group	Control group	*P* value
European American children	*N*	45	57	
	Age, y (mean ± SD)	6.7 ± 0.6	6.8 ± 0.5	.22
	Boys (%)	57.8	59.6	.85
	BMI *z* score, (mean ± SD)	1 ± 1.3	0.5 ± 1.3	.11
	Obese (%)	35.6	21.1	.03
	OAHI, episodes/h	6.8 ± 8.2	0.5 ± 0.3	<.01
Greek children	*N*	64	47	
	Age, y (mean ± SD)	5.9 ± 2.4	6.9 ± 2.6	.03
	Boys,%	60.9	51.1	.3
	BMI z score (mean ± SD)	0.6 ± 1.5	0.2 ± 1.3	.11
	Obese,%	31.3	17	.09
	OAHI, episodes/h	9.3 ± 10.9	0.7 ± 0.2	<.01

Abbreviations: y, years; SD, standard deviation; BMI, body mass index; OAHI, obstructive apnea–hypopnea index; h, hour.

Table 2
Distribution of the C-reactive protein (CRP) 1444C/T, CRP −717T/C, CRP 1861C/T, CRP 1919A/T, IL-6-174G/C, and IL-6 597G/A variants (single nucleotide polymorphisms) among the case group and control group for European American children (A) and Greek children (B). The *P* values for testing the association between genotype distribution of each polymorphism and susceptibility to obstructive sleep apnea are shown.

SNPs	Genotype	Case group	Control group	P value
(A) *European American children*		N = 45	N = 57	
CRP 1444C/T	CC	19	46	<.01
(rs1130864)	CT	19	7	
	TT	7	4	
CRP −717T/C	TT	26	40	.10
(rs2794521)	TC	16	17	
	CC	3	0	
CRP 1861C/T	CC	25	33	.97
(rs1205)	CT	16	19	
	TT	4	5	
CRP 1919A/T	AA	23	44	.02
(rs1417938)	AT	17	9	
	TT	5	4	
IL-6-174G/C	GG	37	36	.03
(rs1800795)	GC	4	17	
	CC	4	4	
IL-6 597G/A	GG	38	38	.08
(rs1800797)	GA	4	15	
	AA	3	4	
(B) *Greek children*		N = 64	N = 47	
CRP 1444C/T (rs1130864)	CC	40	35	.41
	CT	20	10	
	TT	4	2	
CRP −717T/C (rs2794521)	TT	32	25	.21
	TC	27	14	
	CC	5	8	
CRP 1861C/T (rs1205)	CC	30	20	.49
	CT	29	20	
	TT	5	7	
CRP 1919A/T (rs1417938)	AA	41	30	.99
	AT	18	13	
	TT	5	4	
IL-6-174G/C (rs1800795)	GG	37	22	.51
	GC	23	21	
	CC	4	4	
IL-6 597G/A (rs1800797)	GG	38	25	.65
	GA	22	17	
	AA	4	5	

Abbreviations: SNP, single nucleotide polymorphisms; CRP, C-reactive protein.

For the CRP 1919A/T variant, the allele contrast and the model-free approach produced significant results (OR, 2.45 [95% CI, 1.23–4.85] and OR_G, 2.76 [95% CI, 1.26–6.03], respectively) (Table 3). The OR of the additive model did not show significance (unadjusted or adjusted), whereas the OR of the codominant model showed significance. However, the OR of the additive model was significant after correcting for deviation from HWE (OR, 6.97 [95% CI, 1.70–28.7]), whereas the codominant OR was nonsignificant (OR, 1.79 [95% CI, 0.86–3.72]). Hence the mode of inheritance was considered to be nondominance of allele T ($h = 0$ [Table 4]).

For the IL-6-174G/C variant, significance was shown for the codominant model (OR, 0.23 [95% CI, 0.07–0.74] and $OR_{adjusted}$, 0.21 [95% CI, 0.06–0.81]), indicating recessiveness of allele C ($h = -48.3$) (Table 4).

3.1.2. Greeks

None of the genotype distributions showed significant association with the clinical status (Table 2). In addition, the examined genetic models derived nonsignificant results (Table 3).

3.2. Haplotype analysis

In Table 5, *P* values for testing LD between pairs of variants for the cases and controls are summarized. The distribution of the estimated haplotype frequencies for cases and controls is presented in Table 6. Both Tables include only genetic variants with the controls in HWE.

In European American children, the IL-6-174G/C variant was in LD with the IL-6 597G/A for both cases and controls ($P < .01$) (Table 5). Regarding the CRP −717T/C and CRP 1861C/T haplotypes overall, there was no significant difference between the cases and the controls ($P = .46$) (Table 6). For IL-6-174G/C and IL-6 597G/A haplotypes, there was a marginally significant difference ($P = .06$) (Table 6). This difference was due to the CA haplotype ($P = .03$).

In Greek children, the CRP 1444C/T and CRP 1919A/T variants were in LD ($P < .01$) for both cases and controls, while the CRP 1861C/T and CRP 1919A/T variants were in LD only for the control participants ($P = .02$) (Table 5). Regarding the CRP and IL-6 haplotypes, overall, there was no significant difference between cases and controls ($P = .18$ and $P = .27$, respectively) (Table 6).

4. Discussion

Our study was based on the hypothesis that variation in CRP and IL-6 genes, which are both mediators with a crucial role in immunity and inflammation, may affect the susceptibility to OSA in childhood. Using a multifaceted methodologic approach, it was demonstrated that the CRP SNPs 1444C/T and 1919A/T, as well as the IL-6-174G/C SNP, were related to an increased risk for the presence of OSA in the European American population. Such associations were not identified in the Greek population. These findings in European American children may imply that the IL-6/CRP pathway is causally associated with OSA or alternatively the IL-6/CRP gene variants are tightly linked to the true disease mutations.

Our report is the first genetic association study evaluating the relationship of CRP and IL-6 gene variants with OSA in two different pediatric populations. There are only two other studies assessing the IL-6 gene variants in OSA [21,32]. The majority of published investigations on the genetics of OSA have been mainly focused on adults [33]. Although there are numerous parallels between pediatric and adult OSA, the two conditions seem to be only partially overlapping. In children, symptoms include not only habitual snoring and restless sleep, but also daytime behavioral, mood, and learning problems. In contrast to adults with OSA, daytime sleepiness is not as frequently manifested in young children [1]. Furthermore, the major pathogenetic role of adenotonsillar hypertrophy in pediatric OSA most likely originating from systemic and localized upper airway immunologic and inflammatory processes is nearly absent in adults [34–36].

The inconsistency regarding gene variants that produce significant associations with OSA between European American and Greek populations might reflect differences in genetic background (e.g., ancestral effects), environmental exposure, or disease phenotypes [37]. It is of interest that the CRP SNPs 1444C/T and 1919A/T, risk factors for OSA only in US children in our study, are in LD in both the Greek cases and control group. Nonetheless, the sample size of the two cohorts is small and this inconsistency should be interpreted with caution. To this effect, we should also remark that several other SNPs in the CRP and IL-6 genes have been linked to measurable disease phenotypes including OSA in adults. For example, Larkin et al. [7] reported that rs2808630–a SNP in the 3′ untranslated region of the CRP gene–is associated with OSA in European Americans after our study was initiated. This specific variant has been related to reduced CRP serum levels in non-Hispanic African Americans or does not have an effect on CRP levels in Chinese adults [38,39]. Accordingly, a more comprehensive coverage of CRP and IL-6 genes in future large scale studies appears warranted. Systemic inflammation as reflected by CRP blood levels is an OSA phenotype, which is different in US and Greek children.

Table 3
Odds ratios (ORs) and the corresponding 95% confidence intervals (CIs) for testing the association between C-reactive protein variants (1444C/T, −717T/C, 1861C/T, 1919A/T), IL-6 variants (−174G/C and 597G/A), and obstructive sleep apnea for the allele contrast, the model-free approach (generalized odds ratio) and the additive and codominant models for European American children (A) and Greek children (B).

SNP	Genetic model	OR (95% CI)	*P* value	$OR_{adjusted}$ (95% CI)	*P* value
(A) *European American children*					
CRP 1444C/T (rs1130864)	Allelic	3.82 (1.91–7.63)	<.01		
	Model-free	4.37 (1.96–9.76)	<.01		
	Additive	4.24 (1.10–16.2)	.04	4.50 (1.03–19.7)	.05
		[a]15.8 (3.94–63.43)			
	Codominant	5.26 (1.96–14.29)	<.01	6.25 (2.27–20.0)	<.01
		[a]2.47 (1.17–5.21)			
CRP −717T/C (rs2794521)	Allelic	1.85 (0.91–3.74)	.12		
	Model-free	1.75 (0.80–3.86)	.16		
	Additive	10.7 (0.53–215.6)	.14	NA	
	Codominant	1.30 (0.56–2.94)	.54	1.37 (0.54–3.45)	.50
CRP 1861C/T (rs1205)	Allelic	1.07 (0.57–2.00)	.97		
	Model-free	1.08 (0.53–2.22)	.83		
	Additive	1.06 (0.26–4.34)	.94	0.55 (0.11–2.72)	.46
	Codominant	1.10 (0.49–2.50)	.81	1.11 (0.44–2.78)	.83
CRP 1919A/T (rs1417938)	Allelic	2.45 (1.23–4.85)	.02		
	Model-free	2.76 (1.26–6.03)	.01		
	Additive	2.39 (0.59–9.78)	.23	2.43 (0.54–10.9)	.25
		[a]6.97 (1.70–28.7)			
	Codominant	3.23 (1.27–8.33)	.01	4.00 (1.43–11.1)	.01
		[a]1.79 (0.86–3.72)			
IL-6-174G/C (rs1800795)	Allelic	0.55 (0.26–1.16)	.16		
	Model-free	0.45 (0.19–1.08)	.07		
	Additive	0.97 (0.23–4.29)	.97	0.71 (0.13–3.80)	.69
	Codominant	0.23 (0.07–0.74)	.01	0.21 (0.06–0.81)	.02
IL-6 597G/A (rs1800797)	Allelic	0.50 (0.22–1.10)	.12		
	Model-free	0.42 (0.17–1.07)	.06		
	Additive	0.75 (0.16–3.58)	.72	0.39 (0.06–2.58)	.33
	Codominant	0.27 (0.09–0.89)	.03	0.25 (0.06–0.96)	.04
(B) *Greek children*					
CRP 1444C/T (rs1130864)	Allelic	1.60 (0.79–3.24)	.26		
	Model-free	1.69 (0.77–3.73)	.19		
	Additive	1.75 (0.30–10.1)	.53	1.27 (0.20–7.88)	.80
	Codominant	1.67 (0.70–4.00)	.25	1.79 (4.55–0.70)	.22
CRP -717T/C (rs2794521)	Allelic	0.87 (0.49–1.55)	.74		
	Model-free	0.95 (0.48–1.85)	.88		
	Additive	0.49 (0.14–1.68)	.26	0.49 (0.14–1.73)	.27
		[a]0.70 (0.19–2.52)			
	Codominant	1.72 (0.78–3.85)	.18	1.61 (0.68–3.70)	.28
		[a]0.95 (0.56–1.63)			
CRP 1861C/T (rs1205)	Allelic	0.77 (0.44–1.36)	.46		
	Model-free	0.77 (0.39–1.51)	.45		
	Additive	0.48 (0.13–1.72)	.26	0.44 (0.11–1.75)	.24
	Codominant	1.12 (0.52–2.38)	.77	1.19 (0.54–2.63)	.67
CRP 1919A/T (rs1417938)	Allelic	0.97 (0.51–1.85)	.94		
	Model-free	0.98 (0.48–2.03)	.96		
	Additive	0.92 (0.23–3.70)	.90	0.65 (0.15–2.89)	.57
	Codominant	1.02 (0.44–2.38)	.96	1.03 (0.43–2.50)	.95
IL-6-174G/C (rs1800795)	Allelic	0.72 (0.40–1.30)	.34		
	Model-free	0.67 (0.33–1.34)	.26		
	Additive	0.60 (0.14–2.62)	.49	0.48 (0.10–2.30)	.36
	Codominant	0.69 (0.32–1.49)	.35	0.69 (0.31–1.56)	.38
IL-6 597G/A (rs1800797)	Allelic	0.76 (0.42–1.39)	.46		
	Model-free	0.76 (0.38–1.53)	.44		
	Additive	0.53 (0.13–2.15)	.37	0.38 (0.08–1.73)	.21
	Codominant	0.93 (0.42–2.04)	.85	0.99 (0.44–2.27)	.99

Abbreviations: SNP, single nucleotide polymorphisms; NA, not applicable; OR, odds ratio; CI, confidence interval; CRP, C-reactive protein.
The ORs adjusted for age, gender and obesity and ORs corrected for deviation from Hardy–Weinberg equilibrium (HWE) also are shown.
[a] Corrected for deviation from HWE.

Tauman et al. [10] studied US children who underwent PSG evaluation for OSA and measurement of morning CRP levels. Significant associations between PSG indices and CRP levels were identified even after adjustment for obesity. However, a subsequent study by Kaditis et al. [11] did not replicate this association in a cohort of Greek children with OSA. Significant reductions in CRP levels following resolution of OSA have been reported, thereby providing further evidence that intermittent upper airway obstruction during sleep induces elevations in blood CRP concentrations, independent of obesity in some populations of children around the world [19,40–42].

The CRP gene is located at the proximal long arm of chromosome 1 in the 1q23.2 region, and it is composed of one intron separating two exons [43]. Approximately 40 SNPs have been recognized [44]. The most commonly reported are −757T/C, −717T/C, −409G/A, and −390C/T/A in the promoter; 29A/T in the

Table 4
The *h*-index and the respective mode of inheritance for the significant variants in the European American population.

	h-Index	Mode of inheritance
CRP 1444C/T	1.15	Dominance of allele T
(rs1130864)	[a]0.32	Recessiveness of allele T
CRP 1919A/T	1.35	Dominance of allele T
(rs1417938)	[a]0.0	Nondominance of allele T
IL-6-174G/C (rs1800795)	−48.3	Recessiveness of allele C

Abbreviation: CRP, C-reactive protein.
[a] Corrected for deviation from the Hardy–Weinberg equilibrium.

intron; 1059G/C in exon 2; 219C/A and 1444C/T in the 3′ untranslated region; and 1846C/T and 2911C/G in the 3′ flanking region. All these polymorphisms are related to the CRP blood levels [45].

IL-6 is an essential mediator of the acute phase response, and its gene is located to 7p15 and consists of five exons and four introns [46]. Polymorphisms in the promoter region of the IL-6 gene may result in interindividual variation in transcription and expression. Three polymorphisms have been reported in the 5′ flanking region of the gene promoter, −174G/C, −572G/C and −597G/A, which appear to affect IL-6 transcription and to be related to higher cytokine plasma levels [47]. The most studied one, the −174G/C, has been associated with increased risk for cardiovascular events and related risk factors, such as CRP, fibrinogen, and hypertension [48,49]. Since CRP blood levels are influenced by gene variants [45], we speculate that the association between OSA and the SNPs IL-6-174G/C and CRP 1444C/T might explain the higher synthesis of CRP in European American children with OSA compared to Greek children with the disorder.

It has been reported that polymorphisms might be in LD and their interaction within haplotypes can be a major determinant of disease susceptibility [50]. The haplotype analysis approach is expected to be more powerful than single-marker analysis, due to the ancestral structure incarcerated in the distribution of haplotypes. In our report, there was only a marginally significant difference in the frequencies of the IL-6-174G/C & 597G/A haplotype in the European American cohort only.

In testing statistically the association between CRP or IL-6 gene variants and susceptibility to OSA, the χ^2 test for trend was applied followed by the allele contrast and the OR_G. The allele contrast tests the allele-specific association and provides more power in identifying an association, as it doubles the number of observations [51,52]. The OR_G quantifies the magnitude of an association allowing estimation of the overall genetic risk effect. It is a model-free approach which utilizes the complete genotype distribution without merging genotypes [14]. In our study, OR_G was adopted

Table 5
The *P* values for testing linkage disequilibrium between pairs of variants in case group and control group.

	Pairs of variants	Case group *P* value	Control group *P* value
European American children	CRP −717T/C vs 1861C/T	.06	.50
	IL-6-174G/C vs 597G/A	<.01	<.01
Greek children	CRP 1444C/T vs 1861C/T	.20	.13
	CRP 1444C/T vs 1919A/T	<.01	<.01
	CRP 1861C/T vs 1919A/T	.48	.02

Abbreviation: CRP, C-reactive protein.

Table 6
Estimated haplotype frequencies for the C-reactive protein and IL-6 variants in the Hardy–Weinberg equilibrium for European American children (A) and Greek children (B). The P values for comparing each haplotype between case group and control group, and the P value for the overall comparison of haplotypes between cases and control participants are shown.

(A) European American children				
Haplotype	Haplotype frequency		*P* value	*P* value overall
CRP −717T/C and 1861C/T	Case group	Control group		
TC	.597	.648	.34	.46
TT	.195	.202	.88	
CC	.169	.133	.36	
CT	.038	.016	.21	
Haplotype IL-6-174G/C and 597G/A				
GG	.896	.833	.10	.06
CG	.026	.012	.33	
CA	.078	.155	.03	
(B) Greek children				
Haplotype	Haplotype frequency		*P* value	*P* value overall
CRP 1444C/T and 1861C/T and 1919A/T	Case group	Control group		
CCA	.031	.075	.143	.18
CCT	.445	.415	.651	
CTA	.008	.001	.390	
CTT	.297	.362	.308	
TTA	.180	.149	.544	
TTT	.039	.001	.053	
Haplotype IL-6-174G/C and 597G/A				
GG	.758	.681	.204	.27
CG	.008	.032	.182	
CA	.234	.277	.474	
GA	.001	.011	.242	

Abbreviation: CRP, C-reactive protein.

because there is no a priori knowledge of the genetic model. Furthermore, OR_G is powerful even in the absence of HWE [27]. After the presence of an association was demonstrated, the mode of inheritance was tested using the h-index, which is a function of the only two independent genetic models: the additive and the codominant [12,13].

No adjustment for multiple comparisons has been performed, as each variant has been tested independently, i.e., we did not make a general null hypothesis that all variants were not associated with OSA simultaneously [53–55]. In addition, all tested comparisons were not independent of each other, and a clear structure in the multiple tests was missing; hence an appropriate multiple test adjustment is impossible. Furthermore, an adjustment for multiple tests should be avoided, as the interpretation of a finding depends on the number of the other tests performed. In particular, there is no need to adjust for the comparisons which are based on the additive and codominant models because the contrasts are orthogonal [29].

In the European American population, control participants did not conform to the HWE for two variants and for one variant in the Greek population, indicating genotyping errors, population stratification, and selection bias in the recruitment of control participants [29]. In population terms, lack of HWE implies existence of migration, selection, mutation, and absence of random mating; however, their impact in candidate-gene studies is limited. An adjustment for deviations from HWE was performed to avoid overestimation of the genetic risk effects [29].

Although significant associations were detected in our study, the size was relatively small, and thus the results should be interpreted with caution. However, it is well-known that candidate-gene studies lack power to detect weak genetic risk effects of common variants. For example, to achieve power of >80% for detecting a modest genetic risk (OR, 1.2) of a variant with prevalence equal to 10%, a sample size of more than 10,000 participants is needed, which is hard to achieve by one or two institutions [56]. Nevertheless, findings of our study could be pooled in a future meta-analysis of multiple studies, providing more power to detect significant associations [29,56]. Furthermore, we used a rather stringent approach for selection of candidate SNPs such as to accommodate the relatively small cohort sizes, even though less stringency approaches might have been desirable [57].

The multifactorial etiology of common disorders involving complex epistatic and gene-environment interactions reduces the likelihood that a single type of studies such as gene-candidate association studies could provide conclusive inferences. An integrative approach combining different kinds of genetic data analysis (i.e., genomic convergence of genetic association studies, genome-wide association studies, genome scans, and microarrays) may be more effective in identifying valid genetic loci [58]. In the era of genomic convergence, any genetic association study could contribute to the elucidation of etiology of complex diseases such as childhood OSA by providing more genetic data.

5. Conclusion

This is the first genetic association study evaluating CRP and IL-6 gene variants in childhood OSA. Certain SNPs in the CRP and IL-6 genes are related to an increased risk for upper airway dysfunction during sleep in European American but not Greek children. Our results might account for the reported discrepant CRP levels among the two populations in the context of pediatric OSA; further, the described findings reinforce the need for additional association studies to clarify the role of the CRP gene in the pathogenesis of childhood OSA and to provide more genetic data for further genomic convergence applications.

Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2013.08.795.

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